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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors: Kivirikko and Pihlajaniemi Assignee: FibroGen, Inc.

Title: SYNTHESIS OF HUMAN PROCOLLAGENS AND COLLAGENS IN RECOMBINANT DNA SYSTEMS

U.S. Serial No.: Not yet assigned Filing Date: Concurrently herewith

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Assistant Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

Please consider the above-identified application in view of the following amendments and remarks.

IN THE CLAIMS

Please cancel claims 1-23 without prejudice. Please enter new claims 24-57 as follows:

24. A recombinant host cell comprising:
 - (a) at least one transfected polynucleotide sequence encoding a human procollagen or collagen or subunit or chain thereof; and
 - (b) at least one transfected polynucleotide sequence encoding prolyl 4-hydroxylase or a subunit of prolyl 4-hydroxylase or a fragment thereof having prolyl 4-hydroxylase activity.

25. The host cell of claim 24, wherein the host cell is a eukaryotic cell.
26. The host cell of claim 24, wherein the host cell is an insect cell.
27. The host cell of claim 24, wherein the host cell is a yeast cell.
28. The host cell of claim 27, wherein the yeast cell is *Pichia pastoris*.
29. The host cell of claim 27, wherein the yeast cell is *Hansenula polymorpha*.
30. The host cell of claim 27, wherein the yeast cell is *Saccharomyces cerevisiae*.
31. The host cell of claim 24, wherein the host cell is a mammalian cell.
32. The host cell of claim 24, wherein the host cell is a non-human cell.
33. The host cell of claim 24, wherein the host cell is a plant cell.
34. The host cell of claim 24, wherein the host cell is a prokaryotic cell.
35. The host cell of claim 34, wherein the prokaryotic cell is a bacterial cell.
36. The host cell of claim 35, wherein the bacterial cell is *E. coli*.
37. A method of producing recombinant human procollagen or collagen, the method comprising:
 - (a) culturing a cell comprising at least one transfected polynucleotide sequence encoding a human procollagen or collagen or subunit or chain thereof, and at least one transfected polynucleotide sequence encoding prolyl 4-hydroxylase or a subunit of prolyl 4-hydroxylase or a fragment

thereof having prolyl 4-hydroxylase activity, under conditions suitable for expression; and

- (b) recovering the procollagen or collagen or subunit or chain thereof.

38. A recombinant host cell comprising:

- (a) at least one transfected polynucleotide sequence encoding a human procollagen or collagen or subunit or chain thereof selected from the group consisting of type IV, type V, type VI, type VII, type VIII, type IX, type X, type XI, type XII, type XIII, type XIV, type XV, type XVI, type XVII, type XVIII, and type XIX procollagen or collagen; and
- (b) at least one transfected polynucleotide sequence encoding prolyl 4-hydroxylase or a subunit of prolyl 4-hydroxylase or a fragment thereof having prolyl 4-hydroxylase activity.

39. The host cell of claim 38, wherein the host cell is a eukaryotic cell.

40. The host cell of claim 38, wherein the host cell is an insect cell.

41. The host cell of claim 38, wherein the host cell is a yeast cell.

42. The host cell of claim 41, wherein the yeast cell is *Pichia pastoris*.

43. The host cell of claim 41, wherein the yeast cell is *Hansenula polymorpha*.

44. The host cell of claim 41, wherein the yeast cell is *Saccharomyces cerevisiae*.

45. The host cell of claim 38, wherein the host cell is a mammalian cell.

46. The host cell of claim 38, wherein the host cell is a non-human cell.

47. The host cell of claim 38, wherein the host cell is a plant cell.

48. The host cell of claim 38, wherein the host cell is a prokaryotic cell.
49. The host cell of claim 48, wherein the prokaryotic cell is a bacterial cell.
50. The host cell of claim 49, wherein the bacterial cell is *E. coli*.
51. A method of producing recombinant human procollagen or collagen, the method comprising:
 - (a) culturing a cell comprising at least one transfected polynucleotide sequence encoding a human procollagen or collagen or subunit or chain thereof selected from the group consisting of a type IV, type V, type VI, type VII, type VIII, type IX, type X, type XI, type XII, type XIII, type XIV, type XV, type XVI, type XVII, type XVIII, and type XIX procollagen or collagen, and at least one transfected polynucleotide sequence encoding prolyl 4-hydroxylase or a subunit of prolyl 4-hydroxylase or fragment thereof having prolyl 4-hydroxylase activity, under conditions suitable for expression; and
 - (b) recovering the procollagen or collagen or subunit or chain thereof.
52. A method of generating a recombinant host cell for the production of recombinant human procollagen or collagen, the method comprising:
 - (a) transfecting at least one polynucleotide sequence encoding a human procollagen or collagen or subunit or chain thereof into a cell; and
 - (b) transfecting at least one polynucleotide sequence encoding prolyl 4-hydroxylase or a subunit of prolyl 4-hydroxylase or fragment thereof having prolyl 4-hydroxylase activity into the cell.

53. A recombinant human procollagen or collagen produced by the method comprising:
- (a) culturing a cell comprising at least one transfected polynucleotide sequence encoding a human procollagen or collagen or subunit or chain thereof, and at least one transfected polynucleotide sequence encoding prolyl 4-hydroxylase or a subunit of prolyl 4-hydroxylase or a fragment thereof having prolyl 4-hydroxylase activity, under conditions suitable for expression; and
 - (b) recovering the procollagen or collagen or subunit or chain thereof.
54. A composition comprising recombinant human procollagen or collagen or a subunit or chain thereof of one type, essentially free from any other type of procollagen or collagen.
55. The composition of claim 54, wherein the human procollagen or collagen or subunit or chain thereof is selected from the group consisting of type I, type II, type III, type IV, type V, type VI, type VII, type VIII, type XIX, type X, type XI, type XII, type XIII, type XIV, type XV, type XVI, type XVII, type XVIII, and type XIX procollagen or collagen.
56. A recombinant host cell for the production of one type of recombinant human procollagen or collagen essentially free from any other type of procollagen or collagen, the host cell comprising:
- (a) at least one transfected polynucleotide sequence encoding a human procollagen or collagen or subunit or chain thereof; and
 - (b) at least one transfected polynucleotide sequence encoding prolyl 4-hydroxylase or a subunit of prolyl 4-hydroxylase or a fragment thereof having prolyl 4-hydroxylase activity.

57. A method of producing recombinant human procollagen or collagen of one type, free of any other type of procollagen or collagen, the method comprising:
- (a) culturing a cell comprising at least one transfected polynucleotide sequence encoding a human procollagen or collagen or subunit or chain thereof, and at least one transfected polynucleotide sequence encoding prolyl 4-hydroxylase or a subunit of prolyl 4-hydroxylase or a fragment thereof having prolyl 4-hydroxylase activity, under conditions suitable for expression; and
 - (b) recovering the procollagen or collagen or subunit or chain thereof.

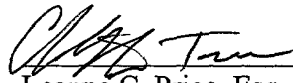
REMARKS

Justification for the amendments to the claims is as follows. Support for new claims 24-57 is found in claims 1-23 as originally filed and generally throughout the specification. In particular, support for the host cells of new claims 24-36, 38-50, and 56 is found in claims 21-23 as originally filed, as well as throughout the specification, for example, at page 11, lines 1 through 16, at page 22, line 18 to page 27, line 33, and at page 29, lines 3 through 10. Support for the procollagen or collagen subunit or chain recited in new claims 24, 37, 38, and 51-57 is found throughout the specification, for example, at page 14, lines 3 through 6, at page 8, lines 17 through 19, and at page 20, lines 1 through 3 and 9 through 12. Support for the prolyl 4-hydroxylase subunits and fragments thereof recited in new claims 24, 37, 38, 51-53, and 56-57 is found generally throughout the specification, for example, at page 20, lines 11 through 26, and in Example 7, and in, e.g., claims 1, 5-7, 11-13, 17, 18, and 21 as originally filed. The methods of new claims 37, 51, 52 and 57 find support throughout the specification and in claims 1-12 as originally filed. No new matter is added by any of the above amendments.

If there are any questions regarding the present communication or the above-referenced application, please call Applicants' Attorney at 650-866-7254.

Respectfully submitted,
FibroGen, Inc.

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